Determining field characters to distinguish the White-footed mouse (*Peromyscus leucopus)* from the Deer mouse (*P. maniculatus)* in Delaware County, Indiana through salivary amylase and PCR genetic analysis

An Honors Thesis (HONRS 499)

by

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Determining field characters to distinguish the White-footed mouse (*Peromyscus leucopus)* from the Deer mouse (*P. maniculatus)* in Delaware County, IN through salivary amylase and PCR genetic analysis

Principle Investigator: Leah Heady

Faculty Advisor: Dr. T. Carter, Department of Biology

Acknowledgements: Laura Smith did lab work for my project for an Honors fellowship under Dr. H. Bruns, and without them this work could not have been completed. I also thank my volunteer field assistants Lindsey Hayter, Cathy Janiczak, Timothy Jedele, Julia Nawrocki, Stephanie Rutan, Jennifer Strong, Allison Treadway, and Zac Warren. My faculty advisor Dr. Carter was also an invaluable resource.

Funding: Funded through BSU's Department of Biology Ecological Research Grant ($500) and through a BSU's Honors' College fellowship.

Synopsis:

For this research I applied for and received a $500 Ball State Ecological Research grant through BSU's Department of Biology. For a part of that grant I developed a project proposal to receive the grant, an interim report halfway through the project, and I presented a poster at BSU's Undergraduate Research Symposium. These components are included before my final research paper.

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Project Proposal: Determining field characters to distinguish *Peromyscus leucopus* from *P. maniculatus* in Delaware County, IN

Principle Investigator: Leah Heady

Faculty Mentor: Dr. Carter

ABSTRACT

The white-footed mouse (*Peromyscus leucopus*) and deer mouse (*P. maniculatus*) are difficult to distinguish in the field due to a large amount of within-species variation and considerable overlap in morphology between species. Coloration is very similar and body measures vary geographically, so in order to distinguish the two species in our area a model needs to be developed. Being able to accurately distinguish to species in the field could be important for many future ecological studies in our region. In order to develop this model, mice of the two species will be captured on several Ball State field properties using baited Sherman live traps in transects. When mice are captured they will be measured, have a cheek swab taken, and then the mice will be marked and released. The cheek swab will be taken back to the lab to run electrophoresis of salivary amylase in order to unambiguously identify the animal to species. Discriminant function analysis will then be used to see what measure, or combination of measures can best identify the species in the field. This model will then be tested on newly captured mice.

INTRODUCTION

White-footed mice (*Peromyscus leucopus*) and deer mice (*P. maniculatus*) are difficult to distinguish in the field. Being able to distinguish in the field alleviates the need to kill the mice or to have to run more costly and time-consuming tests in the lab. There is a lot of within-species variation as well as similar coloration and a considerable overlap in measurements of morphological characteristics between the two species in the Eastern United States (Lackey et al 1985, Sternburg and Feldhamer 1997, Bruseo et al 1999, Laerm and Castleberry 2007). Mensural characteristics vary geographically, even on a county level (Choate et al 1979). Both species have brownish dorsal pelage, white ventors, and white feet. *P. maniculatus* usually have a shorter tail length, shorter hind foot length, and shorter ears than *P. leucopus* (Choate et al 1979, Mumford and Whitaker 1982, Lackey et al 1985, Kamler et al 1998, Laerm and Castleberry 2007), but in some places these traits are reversed (Laerm et al 2007). *P. maniculatus* possess distinctly bicolored tails while *P. leucopus* have indistinctly bicolored tails. The tails of *P. maniculatus* are more densely furred than *P. leucopus* and *P. maniculatus* tails have a more pointed tip, akin to a sharpened pencil (Mumford and Whitaker 1982). *P. leucopus* are also more "bug-eyed" than *P. maniculatus* (Aquadro and Patton 1980). Electrophoresis of salivary amylase (Aquadro and Patton 1980) is one of a variety of genetics tests available to unambiguously discriminate between the two species. Electrophoresis of salivary amylase is relatively inexpensive, provides unambiguous results, and does not require killing the animal. This test has been used widely to distinguish between

the two species (Aquadro and Patton 1980, Feldhamer et al 1983, Rich et al 1996, Sternburg and Feldhamer 1997, Bruseo et al 1999). This project will provide me with the opportunity to expand my field research skills and supplement my knowledge with a hands-on learning experience. This project will also fulfill my honors thesis requirement

METHODS

I will use baited Sherman live traps in transects to capture the mice in several of Ball State's field properties. I will then measure each mouse, using mice that are caught early on in the study to determine what measurements to record. I will measure things like tail length, body length, weight, ear height, and hind foot length. I would also like to see if the skull characteristics that can be used to distinguish the species can be measured on a live animal and still provide helpful insight to species identification. I will collect a sample of salivary amylase by swabbing the mouse's cheek. The mouse will be marked and released. The samples will then be taken back to the lab of Dr. Bruns and electrophoresis of salivary amylase will be run on them to identify to species. I may use preserved specimens from the biology department of local origin and use skull morphology (Choate et al 1979, Lackey et al 1985, Reed et al 2004) or genetic testing using species-specific primers (Tessier et al 2004) to confirm species identification and then use the recorded measurements that are provided with each specimen. I will use discriminant function analysis to develop a model for correctly identifying each species. I will test my model on newly captured mice or specimens from the biology collection that were not previously used, verifying using either skull

characters or a genetic test. The genetics component of this project will be conducted in the lab of Dr. Bruns. Dr. Bruns has agreed to assist and support this project and has an undergraduate student that will assist with the project and take the primary role in overseeing the genetics aspect of this project.

EXPECTED RESULTS AND SIGNIFICANCE OF RESEARCH

I am expecting to develop a model to distinguish between *P. maniculatus* and *P. leucopus* in the field. This model would alleviate the need for running time consuming and costly genetics tests or having to kill the animal to identify it using skull morphology in our area. Being able to readily identify *Peromyscus* in our region could benefit many ecological studies in the future both here on campus and in the surrounding area as well as provide a useful tool for other biologists in the northern Indiana area. I will create a paper and am anticipating a presentation of my results.

BUDGET

$500

BUDGET JUSTIFICATION

Most of the supplies I need are all consumables that are needed in order to capture the mice and to run the electrophoresis of salivary amylase. These two steps are necessary in order to unambiguously determine the species of the mice to create my model. I also need to check and move the traps to different locations so travel expenses are a necessary incurrence. Exact costs are difficult

to determine as the exact genetic method is not commonly used today because of its relative crudeness; however this test is perfectly suited for our needs and is relatively inexpensive. Dr. Bruns has commented that $500 should be sufficient to purchase the supplies for the lab portion of the project.

LITERATURE CITED

Aquadro, C.F. and J. C. Patton. 1980. Salivary amylase variation in *Peromyscus*: Use in species identification. Journal of Mammalogy 61:703-707.

Bruseo, J. A., S. H. Vessey, and J. S. Graham. 1999. Discrimination between *Peromyscus leucopus noveboracensis* and *Peromyscus maniculatus nubiterrae* in the field. Acta Theriologica 44:151-160.

Choate, J. R., R. C. Dowler, and J.E. Krause. 1979. Mensural discrimination between *Peromyscus leucopus* and *P. maniculatus* (Rodentia) in Kansas. Southwestern Naturalists 24:249-258.

Feldhamer, G. A., J. E. Gates, and J. H. Howard. 1983. Field identification of *Peromyscus maniculatis* and *P. leucopus* in Maryland: reliability of morphological characteristics. Acta Theriologica 27:417-423.

Kamler, J. F. et al. 1998. Variation in morphological characters of the white-footed mouse (*Peromyscus leucopus*) and the deer mouse (*P. maniculatus*) under allotropic and syntopic conditions. American Midland Naturalist 140:170-179.

Lackey, J. A, D.G. Huckaby, and B. G. Ormiston. 1985. *Peromyscus leucopus*. Mammalian Species 247:1-10.

Laerm, J. and S. B. Castleberry. 2007. "White-footed mouse: *Peromyscus leucopus*." The land manager's guide to mammals of the south. Ed. M. K. Trani et al. The Nature Conservancy, Southeastern Region, and The U.S. Forest Service, Southern Region. 332-336.

Mumford, R. E. and J. O. Whitaker 1982. "Deer mouse: *Peromyscus maniculatus*" Mammals of Indiana. Indiana Univ. Press, Bloomington. 310-322.

Reed et al. 2004. Using morphologic characters to identify Peromyscus in sympatry. American Midland Naturalist 152:190-195.

Rich, S. M. et al. 1996. Morphological differentiation and identification of *Peromyscus leucopus* and *P. maniculatus* in northeastern North America. Journal of Mammalogy 77:985-991

Sternburg, J. E. and G. A. Feldhamer. 1997. Mensural discrimination between sympatric *Peromyscus leucopus* and *P. maniculatus* in southern Illinois. Acta Theorologica 42:1-13.

Tessier, N., S. Noel and F. Lapointe. 2004. A new method to discriminate the deer mouse (*Peromyscus maniculatus*) from the white-footed mouse (*Peromyscus leucopus*) using species-specific primers in multiplex PCR. Canadian Journal of Zoology 82:1832-1835.

Interim Report: Determining a method to distinguish species of *Peromyscus* in the field in Delaware County, IN

Peromyscus leucopus and *maniculatus* exhibit geographic variation even between counties (Choate et al 1979). In order to determine a reliable method to distinguish *Peromyscus leucopus* and *Peromyscus maniculatus* in the field in our area I, along with five other undergraduate volunteers, have been live trapping *Peromyscus*. I have been performing a suite of measurements and taking genetic and saliva samples of the mice. These samples are then undergoing PCR (Tessier et al 2004) and/or salivary amylase (Aquadro and Patton 1980) testing performed by an undergraduate in the genetics program.

The measurements I am recording on adult animals are ear length, tail length, body length, and hindfoot length (Hall 1981, Lackey et al 1985, and Kamler et al 1998). I have also chosen to measure the length and width of the head to see if skull differences as determined by Reed et al 2004 between the two species can be detected in the flesh.

Peromyscus have been trapped in a range of habitats from open prairie to closed forest at both Cooper Farm and Christy Woods Field stations. Next semester I will expand my trapping sites to include at least Guinn Woods and Miller Wildlife Area. Field observations of color variation suggest that I have both species as bicoloredness of tail and buffiness of flanks noticeably vary in some individuals.

To date, 68 field-caught specimens have been sampled and 8 samples were taken from preserved museum specimens that had accompanying measurements. Many more samples are expected for next semester now that all equipment is acquired and volunteers are assisting on the project. The final product of this research will be a research paper for my Honors thesis with the goal of publication as well as a presentation.

LITERATURE CITED

Aquadro, C.F. and J.C. Patton. 1980. Salivary amylase variation in *Peromyscus:* Use in species identification. J. Mammal. 61:703-707.

Choate, J.R. et al. 1979. Mensural discrimination between *Peromyscus leucopus* and *Peromyscus maniculatus* (Rodentia) in Kansas. Southwest. Nat. 24:249-258.

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Tessier, N. et al. 2004. A new method to discriminate the deer mouse (*Peromyscus maniculatus*) from the white-footed mouse (*Peromyscus leucopus*) using species-specific primers in multiplex PCR. Can. J. Zool. 82:1832-1835.

Snapshot of poster presented at 2010 BSU's Undergraduate Research Symposium

Determining field characters to distinguish the White-footed mouse (*Peromyscus leucopus*) from the Deer mouse (*P. maniculatus*) in Delaware County, IN through salivary amylase and PCR genetic analysis

Student Investigators:

Field Work: Leah Heady
Laboratory Work: Laura Smith

Faculty Advisors:

Field Work: Dr. T. Carter
Laboratory Work: Dr. H. Bruns

Funding:

Funded through BSU's Department of Biology Ecological Research Grant and BSU's Honors' College.

Ball State University: Department of Biology

Abstract:

The white-footed mouse (*Peromyscus leucopus*) and deer mouse (*P. maniculatus*) are difficult to distinguish in the field due to a large amount of within species variation and considerable overlap in morphology between species. Coloration is very similar and body measures vary geographically, so in order to distinguish the two species in our area a model needs to be developed. Being able to accurately distinguish to species in the field would be important for many future ecological studies in our region. In order to develop this model, mice of the two species were captured on several Ball State University field properties using Sherman live traps in transects. Captured mice were measured, their mouth was swabbed for salivary amylase and then an ear clip was taken for both marking purposes and DNA sample before they were released. In the lab, electrophoresis of salivary amylase was run as well as PCR analysis in order to identify the animal to species. Principal components analysis was then used to see if discriminant function analysis should be used to see what measure, or combination of measures can best identify the species in the field. Unfortunately, a method to determine species in the field was not achieved.

Introduction:

White-footed mice and deer mice are difficult to distinguish in the field. Being able to distinguish in the field also often required to kill the mouse to have to run more costly and time-consuming tests in the lab. There is a lot of within species variation as well as similar coloration and considerable overlap in measurements of morphological characteristics between the two species in the Eastern United States (Lackey et al 1985, Sternburg and Feldhamer 1997, Brunet et al 1999, Laerm and Castleberry 2007). Measures vary very geographically, even on a county level (Choate et al 1979). Both species have brown to dorsal pelage, white venters, and white feet. *P. maniculatus* usually have a shorter tail length, shorter hind foot length, and shorter ears than *P. leucopus* (Choate et al 1979, Mumford and Whitaker 1982, Lackey et al 1985, Kantor et al 1998, Laerm and Castleberry 2007) but in some places these traits are reversed (Laerm et al 2007). *P. maniculatus* possess discretly bicolored tails while *P. leucopus* have indistinctly bicolored tails. The tails of *P. maniculatus* are more densely furred than *P. leucopus* and *P. maniculatus* tails have a more pointed tip, akin to a sharpened pencil (Mumford and Whitaker 1982). *P. leucopus* are also more "bug-eyed" than *P. maniculatus* (Aquadro and Patton 1980). Skull morphology also differs between the two species, although this has not been examined in living specimens (Choate et al 1979, Lackey et al 1985, Reed et al 2004). Electrophoresis of salivary amylase (Aquadro and Patton 1980) is correct? a variety of genetic tools available to discriminate between the two species. Electrophoresis of salivary amylase is relatively inexpensive, has previously been demonstrated to provide unambiguous results, and does not require killing the animal. This test has been used widely to distinguish between the two species (Aquadro and Patton 1980, Feldhamer et al 1983, Richt et al 1996, Sternburg and Feldhamer 1997, Brunet et al 1999). Recently, PCR (polymerase chain reaction) using species-specific primers (Tessier et al 2004) has been used to confirm species identification.

Methods:

Live Sherman live traps placed in transects were used to capture mice in several Ball State University field properties. These were Cooper Farm, Miller Wildlife Area, Gilbert Woods, and Christy Woods, located in Delaware Co., Indiana. These locations included a variety of habitats. The following measurements were then taken on each mouse: body length, tail length, hind foot length (to the nearest start of nail), and skull width (taken directly behind eyes). A sample of salivary amylase was taken for analysis by allowing the mouse to chew on a sterile swab. An ear clip was taken to indicate future recapture as well as to have a genetic sample for PCR. The mouse was then released. The samples were stored at 40° C. DNA was isolated from ear tissue using phenol/chloroform extraction. PCR (See Figure 1) was performed using the following primers: *P. leucopus* - CATTTCTAATAGTGTGCCTC, *P. maniculatus* - GGAATTTATGGGGTCTACATTC. The PCR protocol was as follows: A master PCR mix was made for each sample containing 1.0 µL of dNTP mix, 2.5 µL of Taq buffer, 0.25 µL of $MgCl_2$, 0.5 µL of forward primer, 0.5 µL of reverse primer, 0.5 µL of Taq, 18.75 µL of sterile water, and 2µL of DNA. The 25 µL sample was then put in the PCR machine. The samples were then run for two minutes and 30 seconds at 94°C for denaturing and then 34 cycles on the following temperatures and times: 94°C for 30 seconds, 46°C for one minute, and 72°C for two minutes for annealing, and finally 72°C for ten minutes for extension. The *P. leucopus* species was identified by the presence of a 259 base pair band. Salivary amylase was isolated from each mouse swab using water. The eluted samples were then run on an electrophoresis gradient gel for 35 minutes on a constant voltage of 200 volts (See Figure 2). Protein bands were then observed by coomassie stain and visualized by placing the gel on a black background and taking a photograph. To begin, PCR was run on preserved specimens from Delaware County to determine species (Choate et al 1979, Lackey et al 1985, Reed et al 2004) before running live samples. After determining species, principal components analysis was run to see if a model could even be developed.

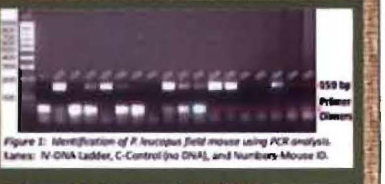

Figure 1: Identification of P. leucopus field mouse using PCR analysis. Lanes: N-DNA Ladder, C-Control (no DNA), and Numbers-Mouse ID.

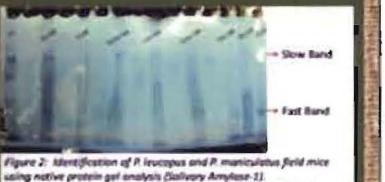

Figure 2: Identification of P. leucopus and P. maniculatus field mice using native protein gel analysis (Salivary Amylase-1). Lanes: PS- Protein Standard, DS-Denatured Swab, S-Swab, DCSa-Denatured Concentrated Salivary Wash, Csa-Concentrated Salivary Wash, DDSa-Denatured Dilute Salivary Wash, Dsa-Dilute Salivary Wash, Numbers- Mouse ID.

Results:

PCR was unsuccessfully run on 16 preserved specimens. 71 mice were captured, and of these, due to field and unknown laboratory error, the species could only be determined for 17 specimens that had accompanying data. Nine were determined to be *P. leucopus* and eight were determined to be *P. leucopus* (assumed *P. maniculatus* due to no *P. maniculatus* bands appearing). Principal components analysis displayed an overlap in the measured variables, so species could not be distinguished by any combination of traits (ear length $p=0.317$, tail length $p=0.879$, body length $p=0.710$, hind foot length $p=0.232$, skull length $p=0.638$, and skull width $p=0.202$). A model could not be developed to distinguish between *P. leucopus* and *P. maniculatus* in the field.

Discussion:

Although a reliable method could not be determined to distinguish these two species in the field, the project still had success. Three students gained valuable field and laboratory experiences as well as experience working both independently and in team. All members of the project learned more about another side of biology through working together be it field or lab work. Future departmental projects can also learn from our experiences.

Conclusions:

One possible explanation for why the two species appeared to be the same is the potential that these two species could hybridize in Delaware County. Future investigations should look to see if these species will interbreed. Another possible explanation is that all samples were from *P. leucopus*. A future investigation that includes known *P. maniculatus* specimens from an area where they are visually distinguishable could remove this speculation. A future investigation could also learn from difficulties encountered on this project in lab and field and produce a large sample size to develop a working model.

Literature Cited:

Final Report: Determining field characters to distinguish the White-footed mouse (*Peromyscus leucopus)* from the Deer mouse (*P. maniculatus)* in Delaware County, IN through salivary amylase and PCR genetic analysis

ABSTRACT

The white-footed mouse (*Peromyscus leucopus*) and deer mouse (*P. maniculatus*) are difficult to distinguish in the field due to a large amount of within-species variation and considerable overlap in morphology between species. Coloration is very similar and body measures vary geographically, so in order to distinguish the two species in our area a model needs to be developed. Being able to accurately distinguish to species in the field could be important for many future ecological studies in our region. In order to develop this model, mice of the two species were captured on several Ball State University field properties using baited Sherman live traps in transects. Captured mice were measured, their mouth was swabbed for salivary amylase and then an ear clip was taken for both marking purposes and a DNA sample before they were released. In the lab, electrophoresis of salivary amylase was run as well as PCR analysis in order to unambiguously identify the animal to species. Principal components analysis was then used to see if discriminant function analysis should be used to see what measure, or combination of measures, can best identify the species in the field. Unfortunately, a method to determine species in the field was not developed.

INTRODUCTION

White-footed mice (*Peromyscus leucopus*) and deer mice (*P. maniculatus*) are difficult to distinguish in the field. Being able to distinguish in the field alleviates the need to kill the mice or to have to run more costly and time-consuming tests in the lab. There is a lot of within-species variation as well as similar coloration and a considerable overlap in measurements of morphological characteristics between the two species in the Eastern United States (Lackey et al. 1985, Sternburg and Feldhamer 1997, Bruseo et al. 1999, Laerm and Castleberry 2007). Mensural characteristics vary geographically, even on a county level (Choate et al. 1979). Both species have brownish dorsal pelage, white ventors, and white feet. *P. maniculatus* usually have a shorter tail length, shorter hind foot length, and shorter ears than *P. leucopus* (Choate et al 1979, Mumford and Whitaker 1982, Lackey et al. 1985, Kamler et al. 1998, Laerm and Castleberry 2007), but in some places these traits are reversed (Laerm et al. 2007). *P. maniculatus* possess distinctly bicolored tails while *P. leucopus* have indistinctly bicolored tails. The tails of *P. maniculatus* are more densely furred than *P. leucopus* and *P. maniculatus* tails have a more pointed tip, akin to a sharpened pencil (Mumford and Whitaker 1982). *P. leucopus* are also more "bug-eyed" than *P. maniculatus* (Aquadro and Patton 1980). Skull morphology also differs between the two species, although this has not been examined in living specimens (Choate et al. 1979, Lackey et al. 1985, Reed et al. 2004). Electrophoresis of salivary amylase (Aquadro and Patton 1980) is one of a variety of genetic based tests available to discriminate between the two species.

Electrophoresis of salivary amylase is relatively inexpensive, has previously been demonstrated to provide unambiguous results, and does not require killing the animal. This test has been used widely to distinguish between the two species (Aquadro and Patton 1980, Feldhamer et al. 1983, Rich et al. 1996, Sternburg and Feldhamer 1997, Bruseo et al. 1999). PCR (polymerase chain reaction), using species-specific primers, (Tessier et al. 2004) and also has been used to confirm species identification.

METHODS

Baited Sherman live traps (H.B. Sherman® small aluminum folding and nonfolding) placed in transects were used to capture mice in several of Ball State University's field properties. These included Cooper Farm, Miller Wildlife Area, Ginn Woods, and Christy Woods all located in Delaware Co., Indiana. These properties included a variety of habitats. Upon capture the following measurements were then recorded for each mouse: body length, tail length, hindfoot length, ear length, skull length (tip of nose to start of spine) and skull width (taken directly behind eyes). A sample of salivary amylase was collected for analysis by allowing the mouse to chew on a sterile swab. An ear clip was taken to indicate future recapture as well as to have a genetic sample for PCR. The mouse was then released. The samples were stored at -42° C. DNA was isolated from ear tissue using phenol/chloroform extraction.

PCR (See Figure 1) was performed using the following primers: *P. leucopus* – CATTTCTAATAGTGTGCCTC, *P. maniculatus* – GGAATTTATGGGTCTACATTC. The PCR protocol was as follows: A master PCR mix was made for each sample containing 1 µL of dNTP mix, 2.5 µL of Taq buffer, 0.25 µL of $MgCl_2$, 0.5 µL of forward primer, 0.5 µL of reverse primer, 0.5 µL of Taq, 18.75 µL of sterile water, and 2 µL of DNA. The 25 µL sample was then put in the PCR machine. The samples were then run for 2 minutes and 30 seconds at 94°C for denaturing and then 34 cycles on the following temperatures and times; 94°C for 30 seconds, 48°C for one minute, and 72°C for two minutes for annealing, and finally 72°C for ten minutes for extension. The *P. leucopus* species was identified by the presence of a 159 base pair band.

Salivary amylase was isolated from each cotton swab using water. The eluted samples were then run on a native protein gradient gel for 35 minutes on a constant 200 volts (See Figure 2). Protein bands were then observed by cooper stain and recorded by placing the gel on a black background and taking a photograph.

Initially, PCR was run on preserved specimens from Delaware County to determine species (Choate et al .1979, Lackey et al. 1985, Reed et al. 2004) before running live samples. After determining species, principal components analysis was run to see if a model could even be developed.

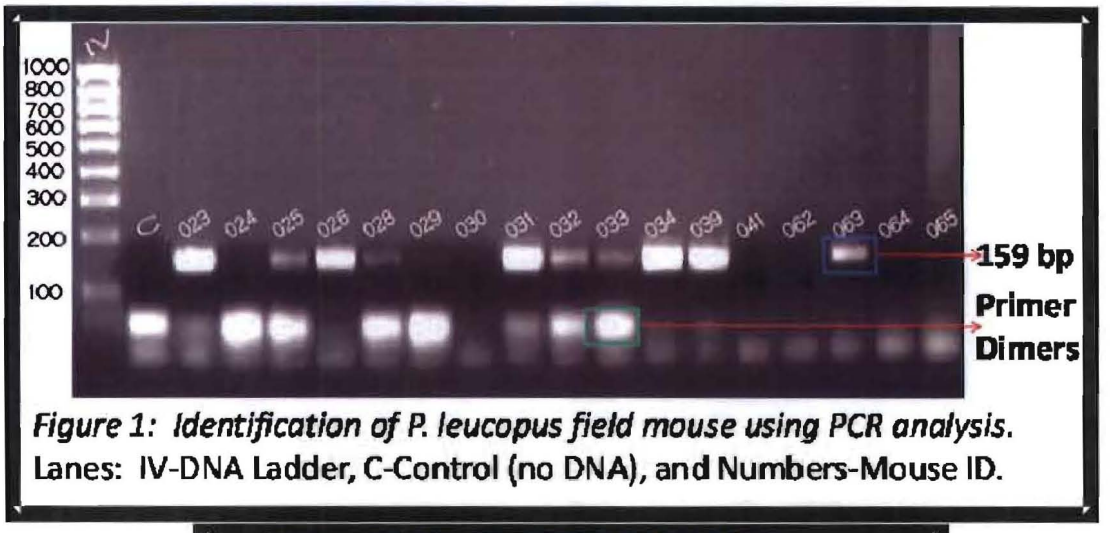

Figure 1: Identification of P. leucopus field mouse using PCR analysis.
Lanes: IV-DNA Ladder, C-Control (no DNA), and Numbers-Mouse ID.

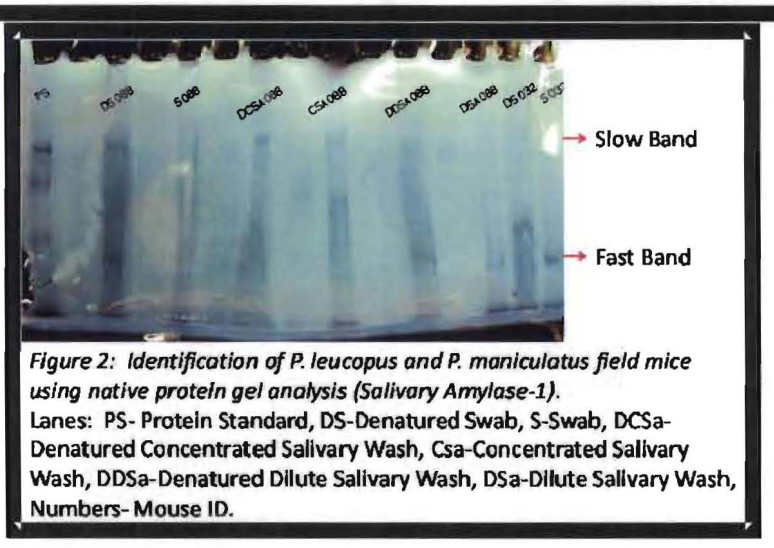

Figure 2: Identification of P. leucopus and P. maniculatus field mice using native protein gel analysis (Salivary Amylase-1).
Lanes: PS- Protein Standard, DS-Denatured Swab, S-Swab, DCSa-Denatured Concentrated Salivary Wash, Csa-Concentrated Salivary Wash, DDSa-Denatured Dilute Salivary Wash, DSa-Dilute Salivary Wash, Numbers- Mouse ID.

RESULTS

PCR was unsuccessfully run on 16 preserved specimens. 71 mice were captured, and of these, due to field and unknown laboratory error, the species could only be determined for 17 specimens that had accompanying data. Nine were determined to be *P. leucopus* and eight were determined to be non *P. leucopus* (assumed *P. maniculatus)* due to no *P. maniculatus* bands appearing. Principal components analysis showed overlap in the measured variables, so species could not be distinguished by any combination of traits (ear length p=0.117, tail length p=0.879, body length p=0.710, hindfoot length p=0.232, skull

length p=0.638, and skull width p=0.782). As a result, a model could not be developed to distinguish between *P. leucopus* and *P. maniculatus* in the field.

DISCUSSION

One possible explanation for why the two species appeared to be the same is the potential that these two species could hybridize in Delaware County. Future investigations should look to see if these species will interbreed. Another possible explanation is that all samples were from *P. leucopus.* A future investigation that includes known *P. maniculatus* specimens from an area where they are visually distinguishable could remove this speculation. A future investigation could learn from difficulties encountered on this project in lab and field and produce a larger sample size that may help to develop a working model.

CONCLUSIONS

Although a reliable method could not be determined to distinguish these two species in the field, the project still had successes. The students gained valuable field and laboratory experience as well as experience working both independently and in teams. All members of the project learned more about another side of biology through working together be it field or lab work. Future departmental projects can also learn from our experiences.

LITERATURE CITED

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Kamler, J. F. et al. 1998. Variation in morphological characters of the white-footed mouse (*Peromyscus leucopus*) and the deer mouse (*P. maniculatus*) under allotropic and syntopic conditions. American Midland Naturalist 140:170-179.

Lackey, J. A, D.G. Huckaby, and B. G. Ormiston. 1985. *Peromyscus leucopus*. Mammalian Species 247:1-10.

Laerm, J. and S. B. Castleberry. 2007. "White-footed mouse: *Peromyscus leucopus*." The land manager's guide to mammals of the south. Ed. M. K. Trani et al. The Nature Conservancy, Southeastern Region, and The U.S. Forest Service, Southern Region. 332-336.

Mumford, R. E. and J. O. Whitaker 1982. "Deer mouse: *Peromyscus maniculatus.*" Mammals of Indiana. Indiana Univ. Press, Bloomington. 310-322.

Reed et al. 2004. Using morphologic characters to identify *Peromyscus* in sympatry. American Midland Naturalist 152:190-195.

Rich, S. M. et al. 1996. Morphological differentiation and identification of *Peromyscus leucopus* and *P. maniculatus* in northeastern North America. Journal of Mammalogy 77:985-991

Sternburg, J. E. and G. A. Feldhamer. 1997. Mensural discrimination between sympatric *Peromyscus leucopus* and *P. maniculatus* in southern Illinois. Acta Theorologica 42:1-13.

Tessier, N., S. Noel, and F. Lapointe. 2004. A new method to discriminate the deer mouse (*Peromyscus maniculatus*) from the white-footed mouse (*Peromyscus leucopus*) using species-specific primers in multiplex PCR. Canadian Journal of Zoology 82:1832-1835.